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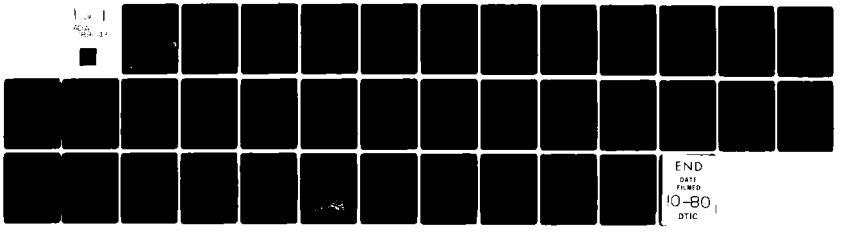

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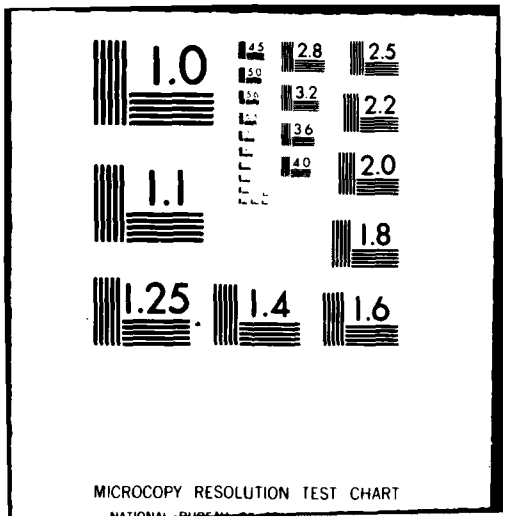
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The effects of PGBx, a newly synthesized polymeric derivative of 15-keto-prostaglandin B_1, were tested in a number of experimental studies in monkeys, dogs and other species. Treatment with PGBx was found to markedly improve survivial after a period of complete circulatory arrest and to restore cardiac function in monkeys and dogs. Pilot studies in attempts to develop a simpler animal or isolated organ test system for PGBx did not

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yield positive results. PGBx has no effect on normal tissues or organs but appears to have unique properties in restoring biological activity following tissue hypoxia or ischemia. Entirely novel experimental approaches are therefore needed to provide the appropriate and critical conditions for demonstrating and quantitating its biological activity.

OFFICE OF NAVAL RESEARCH

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ANIMAL STUDIES IN PGBx

FINAL TECHNICAL REPORT

by

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Philadelphia, Pennsylvania

September 5, 1980

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I. OVERVIEW OF THE STUDIES CONDUCTED:

These studies began on March 1, 1977 and continued through subsequent extensions of the contract to December 31, 1979. The studies conducted could be grouped into two major categories: (a) Definitive studies on the effect of PGBx on recovery following a period of total circulatory arrest produced by ventricular fibrillation which includes extensive studies conducted in monkeys and dogs and (b) An extensive series of pilot studies conducted in a variety of experimental preparations in vivo and in vitro in attempts to develop a simpler test model for the biological effects of PGBx.

The first group of studies indicated that treatment with PGBx had a beneficial effect and were in fact the first demonstration that PGBx had an effect in the entire animal. Extensive studies were therefore made.

The second group of studies for the most part gave megative results in that treatment with PGBx produced no detectable effect and/or the optimal experimental conditions for demonstrating an effect of PGBx were not found.

In this final technical report, a summary of the studies is presented grouped along these lines. This was considered preferable in obtaining an overall view of the project instead of a chronological approach.

Therefore the sequence in which the studies are presented below has no relationship to the sequence in which they were conducted. Furthermore results obtained from one or more separate studies were combined when appropriate.

This report is not intended to be a detailed presentation of the findings which have been or will be reported in scientific journals. Rather the report attempts to present a summary and overview of the findings.

Most of the information contained in this final report has been previously report to the scientific contract monitors in the form of interim progress reports as well as in the several special meetings of contractors working on PGBx organized by ONR.

II. SPECIFIC STUDIES

A. Definitive Studies: Recovery from ventricular fibrillation in the presence of myocardial infarction.

1. Initial Studies on Survival (in monkeys).

The effect of PGBx was tested initially on male Rhesus monkeys (5-9 kg) anesthetized with pentobarbital and subjected to coronary ligation and induced ventricular fibrillation (VF). In tests performed in sequence with intervening period for recovery, intracardiac injections of norepinephrine (NE), cardiac massage (CM), and electrical defibrillation (EDF) were used to restore cardiac function both in controls and experimental animals, but the latter were injected also with 1 mg/kg PGBx. Recovery was established by maintenance of effective blood pressure without exogenous support. In the control group the cumulative survival for fibrillation episodes of 4, 6, 8, and 12 minutes was 60, 40, 31, and 25% respectively. In the PGBx-treated group survival for equivalent periods was 100, 93, 93, and 88% respectively.

In separate studies, African Green monkeys were subjected to single episodes of VF of either 8 or 12 minutes. Combined survival was 36% for the controls, 93% for the PGBx-treated animals. Clearly PGBx radically improved cardiac recovery after circulatory arrest due to VF in the presence of acute myocardial infarction. The results also suggest a synergistic action between norepinephrine and PGBx in achieving such recovery.

A detailed report of these studies has been published (see addendum).

2. Extended studies on cardiac function (in dogs).

Similar studies with PGBx were made in dogs instrumented for a series of measurements of cardiac function. The survival rate of PGBx treated dogs after 8 minutes of VF was 35% in the controls and 86% in the PGBx-treated animals ($P < 0.05$). Since the effect of PGBx in these and the previous studies appeared to be primarily cardiac, additional studies were made to evaluate the effect of PGBx on regional cardiac ischemia. The descending branch of the left anterior coronary was ligated near its origin and measurements of ECG, heart rate, left ventricular pressure (LVP), rate of rise of LVP (dP/dt), blood pressure and cardiac output (CO) (thermodilution techniques) were made. This permitted the independent evaluation of cardiac work (from CO and LVP) as well as the overall left ventricular contractility (max. dP/dt). The ligation was maintained for 30 minutes and then released while all parameters were measured at 10 minute intervals for a period of 60 minutes after release.

Following ligation there was an average decrease in CO of -27 ± 4% and of dP/dt of -20 ± 3% (mean ± S.E.). The treated group received 1 mg/Kg of PGBx intravenously within 2 minutes after release and was compared to the untreated controls. Both groups showed a further decrease in cardiac output 10 minutes after release to approximately -40 to -45% of original control levels. However in the PGBx-treated group CO recovered within the next 10 minutes to pre-release levels where it was maintained for the remaining period of observation. By contrast the control group showed only a partial temporary recovery in CO which was followed by a progressive decline in the remaining period. At the end of 60 minutes, CO in the control group was -45 ± 6% while that of the PGBx-treated group was -30 ± 3% ($P < 0.05$). Cardiac work showed similar changes as CO in each group.

The most striking difference between control and treated animals was observed in the overall left ventricular contractility (max. dP/dt). Following release the control group showed a further decrease of dP/dt to -36 ± 7% (of original preligation levels), which remained at this level with minor variations during the subsequent period of observation. By contrast the PGBx-treated animals did not exhibit on the average a significant decrease in dP/dt following release with values remaining at or above pre-release levels for the remaining period. Thus 60 minutes after release dP/dt levels for the treated animals were at -17 ± 4% as compared to -30 ± 7% for the controls ($P < 0.05$).

In a separate series of studies PGBx was administered at 15 minutes following ligation, as well as after release. In this group of treated animals the progressive decrease of dP/dt during ligation (observed in the controls) was arrested. Similarly as in the previous study there was no further decrease following release.

These results indicate that under the conditions used, PGBx treatment prevents the further deterioration in myocardial function observed during coronary ligation and especially following release. However under the experimental conditions employed PGBx treatment did not restore myocardial function to pre-ligation levels. Nevertheless by preventing further deterioration at the end of the 90 minute period following ligation and release the overall cardiac status (as measured by cardiac output and especially dP/dt) was significantly better in the PGBx-treated animals than in the controls. These findings therefore support the view that PGBx has a favorable effect in the control of ischemic tissue injury.

B. Pilot Studies:

Since the information gained from these studies has not been published and in most cases no further publication is anticipated, the methodologies and findings are presented here in more detail.

1. Hemorrhagic Shock in the Pig.

Two models of hemorrhagic hypovolumic shock were used:

a) Constant pressure shock in which animals were bled to 50 mmHg and maintained at this pressure by further withdrawal or addition of blood for a period of 60 minutes when they were re-infused with the entire blood volume withdrawn ("Wigger's model). With this approach, 16 out of 22 (73%) untreated control animals died at a mean time of 29 minutes after re-infusion. Administration of catecholamines (norepinephrine; NE) did not alter significantly the overall mortality (6 out of 7 or 86%) although it did increase the post-infusion survival time to a mean of 107 minutes. In this model, treatment with PGBx (1 to 2 mg/kg) given in repeated doses during re-infusion in combination with NE did not alter significantly mortality (4 out of 4 or 100%) nor did it prolong the survival time beyond that seen with NE alone (mean of 103 minutes).

However, it was noted that some of these animals were hypoglycemic, therefore a study was made to determine the presence of hypoglycemia in the controls and the effect of glucose infusions. The results were quite variable, some of the animals exhibiting hypoglycemia while others did not. Treatment with intravenous glucose had no consistent effect on overall mortality although it tended to prolong survival time. Because of the variability encountered, this approach was not used for further testing.

b) Fixed volume. The second model involved the withdrawal of fixed volume. Blood volume determinations in 12 animals indicated that withdrawal of blood in amounts corresponding to 3% of body weight was equivalent to 45 to 55% of total blood volume. In this model, a slow bleeding was used (30 minutes) followed by re-infusion when the animals showed spontaneous respiratory arrest and had to be respired artificially. Under these conditions, 2 out of 6 (33%) of the controls died following re-infusion which included pressor support with norepinephrine.

Treatment with PGBx (1-2 mg/kg) during the re-infusion phase in combination with NE did not alter mortality (2 out of 3 or 66%). Hence it appeared that no PGBx activity could be demonstrated in this model either.

In view of the above results and the fact that other agents (such as phenoxybenzamine) were found to have a very striking protective effect in hemorrhagic shock in the pig, further testing with PGBx in these models of hemorrhagic shock in the pig was terminated. Even though a small number of PGBx-treated animals were used in each case, sequential statistical analysis (based onthe results obtained in a larger series of controls and a smaller number of test animals) indicates that the probability an effect of PGBx would be demonstrated in these test models is less than 5% if a large number of animals were used. Therefore, it can be safely concluded that PGBx has no effect on these models. However there is, of course, the possibility that a more elaborate experimental design involving simultaneous glucose administration and/or pretreatment with PGBx and/or different doses or infusions of PGBx, etc. could yield different results.

2. Myocardial Infarction in the Pig.

A series of controls indicated a mortality of 7 out of 12 (58%) after 4 minutes of ventricular fibrillation (VF) in the presence of coronary occlusion and a mortality of 4 out of 6 (67%) after 8 minutes of VF under similar conditions. Preliminary studies in a few animals pretreated with PGBx showed complete recovery after 8 minutes of VF (2 out of 2 animals). These results were consistent with the previous studies in monkeys. However, the primary purpose of these studies was to determine the potential effect of PGBx in altering the contractility of the ischemic segment following occlusion. Control experiments in 8 animals showed an immediate marked decrease in contractility of the ischemic segment following occlusion. Detailed studies with PGBx in 2 animals showed no effect on the contractility of the ischemic segment even after repeated administration of PGBx (1-2 mg/kg/dose).

In view of this observation and the fact that previous preliminary studies conducted at the Unviersity of Pennsylvania in one dog (as reported by Dr. Polis) suggested a definite effect of PGBx on ischemic segment contractility under these conditions, it was jointly decided with Dr. Polis to re-design and conduct these studies in the dog rather than in the pig.

It should be noted that Dr. Polis personally participated in most of the experiments on the pig when PGBx was tested. In the course of these studies, he provided many suggestions for alterations and/or improvement of experimental design and in each case, it was necessary to re-establish the baseline parameters in new series of controls before PGBx was tested. Therefore, even though the animals tested with PGBx in each case were limited, the preliminary results were considered sufficient in making the determination not to proceed with more extensive tests in a

given model based on the broader information available in the controls. As indicated, sequential statistical analysis supports these conclusions.

3. Glucose Tolerance Curves in Monkeys.

Based on Dr. Polis' findings that PGBx has an effect on diabetic mice and certain other results suggesting that hypoglycemia may limit or obscure the potential effect of PGBx, it appeared necessary to test whether PGBx had any effect on the blood glucose tolerance curve.

Detailed studies were made in 9 controls and 3 PGBx-treated monkeys. A standard glucose tolerance test was used involving the intravenous administration of 0.5 gms of gluocse with plasma glucose levels measured at 5, 10, 20, 30, 60 and 120 minute intervals. PGBx (1-2 mg/kg), was administered either before the glucose (pre-treatment) or near the peak of the blood glucose level (10 minutes after I.V. glucose). In control animals, glucose tolerance curves were quite reproducible both between animals and in repeated tests in the same animal. PGBx administered under these conditions in these dose levels had no detectable effect on the blood glucose tolerance curves on either the magnitude of response or rate of removal. In addition, in one animal tests, PGBx had no detectable effect on the insulin response to the glucose tolerance test. Plasma insulin in both control and treated animals was determined with the conventional radio-immunoassay technique.

The results of these studies showed that PGBx has no acute effect on glucose utilization and/or insulin release under these conditions.

4. Hyperglycemia after Epinephrine.

Since a number of preliminary findings suggested that there may be a synergism between PGBx and catecholamines, the effect of PGBx pre- and post-treatment was tested on the hyperglycemic response to epinephrine. In a series of specific studies, a total of nine control and nine PGBx-treated monkeys were tested. PGBx was administered in doses of 1 to 2 mg/kg before or during the hyperglycemic response produced by the intravenous administration of 25, 50, 100 and 200/µg of epinephrine.

In control animals, these doses of epinephrine produced a prolonged hyperglycemic response which was maximal at the 100/µg dose level. At the same time, there was a suppression of insulin secretion as expected and attributed to the known direct inhibitory effect of epinephrine on insulin secretion.

PGBx treatment did not alter the hyperglycemic response to 50 or 100/µg of epinephrine. Neither the magnitude nor the duration of the hyperglycemic response nor the insulin suppression were significantly effected.

With large doses of epinephrine (200/µg), PGBx treatment appeared to prolong the hyperglycemic response. However, these large doses of epinephrine produced significnat pulmonary edema and both PGBx-treated animals did not recover (two out of four controls recovered). Therefore, the potential effect of PGBx on the hyperglycemic response to very large doses of epinephrine could not be tested further without the complications that would have had to be introduced if additional agents were included in the experimental design to prevent or reduce the pulmonary edema.

From these studies, it was concluded that PGBx did not alter the hyperglycemic response of epinephrine at least in concentrations prevailing under physiological or moderate stress conditions.

5. Diabetic Mice.

In view of Dr. Polis' findings of marked effects of chronic administration of PGBx (over several weeks) on a strain of diabetic mice, tests were made to determine whether PGBx treatment would produce any detectable effect on glycosuria and/or blood glucose levels in these animals after short-term treatment (24 hours to 3 days). The aim here was to attempt to develop a rather simple in vivo bioassay for PGBx using this animal model.

Mice of the obese diabetic strain were obtained from the Bar Harbor Laboratories together with corresponding controls from the same supplier.

Five groups (three control and two diabetic) of five mice each were followed for several days regarding urine volume and urinary glucose and creatinine determinations. The diabetic groups have a high glycosuria amounting to concentrations between 60 to 130 mg/ml, while the control animals had less than 2 mg/ml of urinary glucose.

In the first experiment of this series, PGBx was administered subcutaneously in a dose of 5µg/gm once a day for three days into both diabetic and control groups of animals. There was no change in the controls. However, in the diabetic animals, the urinary glucose concentration was decreased to 3 mg/ml (from about 80 mg/ml previously).

This marked change observed in the initial experiments prompted us to undertake the development of a technique for measuring blood glucose levels in mice. Once a satisfactory method was developed after several trials, we found the blood glucose levels of the control mice to be of the order of 93 mg/100 ml, while diabetic mice had levels of 200-300 mg/100 ml.

Administration of PGBx in doses ranging from 2.5 to 12 µg/gm in a series of new groups of animals produced no detectable effects on either control or diabetic mice. At the same time, the effect of PGBx on urinary excretion of glucose was either absent or very minimal. In view of this, we repeated the original experiment several times (measuring urinary glucose) again with generally negative or inconsistent results. Thus, in spite of the original striking findings, we could not reproduce an effect of PGBx on urinary glucose levels in diabetic mice. Nor have we been able to identify any experimental differences in spite of an extensive search for such. One possibility that remains is that for some reason the preparation of PGBx we were using had lost its potency (PGBx from the same batch was used for all the studies in diabetic mice). However, we have no independent evidence to support this possibility since PGBx was kept under conditions previously found adequate for maintaining potency. It is also noteworthy that these experiments were performed in close time sequence.

6. Isolated Perfused Atria.

In collaboration with Dr. Torres and Mr. Bergmann (graduate student), the effect of PGBx was tested in isolated perfused rabbit atria. Isometric force and rate of development of force (dF/dt) were monitored. Administration of PGBx in the perfusion fluid had no effect on the force of contraction or rate of development of force in such preparations under normal conditions of perfusion and oxygenation.

A series of tests were made in preparations subjected to periods of hypoxia by repeated exposure to perfusions in which oxygen was replaced with nitrogen. PGBx in concentrations of 10 µg/ml (added after the anoxic depression) did not effect the rate or ultimate level of recovery of myocardial force or rate of development of force (dF/dt). Preparations that had lost approximately 20%, 45% and 75% of the original level of tension development were tested. Preparations which had lost up to 75% of the original tension level recovered slowly over a period of one to two hours without complete restoration. PGBx had no detectable effect on the rate or magnitude of ultimate recovery. These depressed preparations were found to be responsible to administration of NE (1 to 10 µg/ml) with a positive inotropic effect. Administrations of PGBx did not seem to alter this effect of NE.

These pilot studies suggested that PGBx had no demonstrable effect in such a pareparation and, therefore, no further studies were made. However, it is entirely possible that the conditions and/or the concentrations used were not optimal for the demonstration of an effect of PGBx in this isolated perfused tissue.

7. Isolated Perfused Rat Hearts.

In collaboration with Dr. Schaffer and Mr. Vary (Graduate student), the effect of PGBx was tested in studies in an isolated perfused heart preparation.* These studies involved 88 controls and 81 PGBx perfused rat hearts. Over a concentration range of .25 - 1.0 mg/l no positive effect of PGBx was detected on the degree of mechanical recovery of the perfused hearts.

The working heart apparatus used was a modified version of that described by Neely et al. (Amer. Heart Journal 212:804-814, 1967). The coronary system of the heart was perfused from a reservoir placed 100 cm. above the aortic cannula, while the left atrium received fluid from an atrial reservoir maintained at 13 cm. of water pressure. When the left atrial cannula was open, there was a net ejection of fluid against the 1-0 cm. pressure head. Cardiac output was determined from the sum of the fluid ejected from the aorta and the coronary effluent. Aortic pressure was measured with a Statham P23Gb pressure transducer. Cardiac work was calculated by multiplying output times aortic pressure.

Two parallel circuits with separate aortic and atrial reservoirs were used to permit a rapid exchange of perfusion fluid through the coronary circulation with a minimum of dead space. One circuit could be modified so that the delivery of fluid to the heart was drastically reduced to induce ischemic conditions.

The standard perfusate (maintained at 37°C) is Krebs-Henseleit buffer supplemented with 5 mM glucose and 10^{-3} units/ml insulin and gassed with a 95% O_2: 5% CO_2 mixture to maintain a pH of 7.4. The protocol for a typical experiment begins with decapitation of the rat from which its heart was rapidly removed and perfused within 45 seconds. The heart was subjected to 15 minutes of control perfusion followed by ischemia for 25-45 minutes. The heart was reperfused for 15 minutes. Percent recovery of cardiac work during reperfusion was compared to hearts treated with PGBx either during periods of ischemia and reperfusion or only during the period of reperfusion.

The first group of studies involved subjecting the hearts to 30 to 45 minutes of ischemia. Thirty minute controls recovered 32 ± 3.2% of the original cardiac work while similar hearts treated with 0.5 mg/L PGBx recovered 27.0 ± 3.6%. PGBx (0.5 mg/L)

*Specifics of the methodology can be found in: Vary, T.C., E. T. Angelakos and S. W. Schaffer: Relationship between adenine nucleotide metabolism and irreversible ischemic tissue damage in isolated perfused rat heart. Circulation Res. 45:218-225, 1979.

treated hearts undergoing 45 minutes of ischemia recovered 3.7 ± 1% of the original cardiac work while a similar series of hearts lacking PGBx recovered 12.0 ± 2.2%. Doses of PGBx of .25 mg/L and 1.0 mg/L were also shown to have no beneficial effect on mechanical recovery.

The next group of studies utilized acetate in the buffer. It has been shown that hearts preferentially utilize fatty acids as metabolic energy sources over glucose. Acetate, metabolized as fatty acids, is readily soluble in the Krebs-Henseleit buffer and does not require a carrier protein. Controls perfused with 5 mM glucose and 5 mM acetate and subjected to 30 minutes of ischemia recovered 39.4 ± 4.1%. Similar hearts treated with .5 mg/L PGBx recovered 40.4 ± 4.9% of the original cardiac work.

Control hearts perfused only with 5 mM acetate as the substrate (no glucose) and subjected to 45 minutes of ischemia recovered 38.5 ± 4.9% while a similar series of PGBx treated (0.5 mg/L) hearts recovered slightly lower at 34.2 ± 4.8%.

Another series of studies was based on the idea that perhaps PGBx could only improve the recovery of more severely treated hearts. This was based on the results obtained with monkeys where PGBx was found to improve the survival, after a period of ventricular fibrillation, of hearts previously subjected to coronary ligation. To provide further damage, the isolated rat hearts were subjected to control perfusion and ischemia without glucose or acetate in the buffer and reperfused with glucose and insulin supplemented buffer or glucose, insulin, and PGBx supplemented buffer. PGBx-treated hearts subjected to 25 or 35 minutes of ischemia under these conditions showed no improvement over controls.

In the studies with monkeys, it was found that intracardiac administration of catecholamines during the resuscitation period after fibrillation improved the incidence of recovery in both control and PGBx-treated preparations. In an attempt to reproduce this finding in isolated rat hearts, a series of studies was conducted in which the hearts were subjected to a 30 minute period of ischemia and reperfused in buffer containing epinephrine (5 µg/L) or with buffer containing epinephrine (5 µg/L) and PGBx (.5 mg/L). Hearts reperfused with epinephrine recovered 21.9 ± 3.2% and hearts reperfused with epinephrine and PGBx recovered 27.4 ± 2.1%. In a similar study, hearts were reperfused with normal glucose supplemented Krebs buffer for 15 minutes after which epinephrine was added to the buffer at 5 µg/L or 10 µg/L. Ten minutes later epinephrine (5 or 10 µg/L) and PGBx (.5 or 1.0 mg/L) were added to the buffer. In these studies, no significant change in recovery occurred during PGBx and epinephrine perfusion.

8. Studies in "Diabetic" monkeys.

In connection with another project, twelve African Green monkeys were identified with abnormal glucose tolerance curves and insulin responses to a glucose load similar to those observed in moderate diabetes in man. In view of the results obtained with PGBx by Dr. Polis in the diabetic strain of mice, we examined the effect of single doses of PGBx on the glucose tolerance responses of these animals. The protocol was the same as that utilized for testing the effects of PGBx on the glucose tolerance responses of normal monkeys (see item #3 above). In these pilot studies PGBx was found to be without any detectable effect. These results are similar to those observed in diabetic mice (see item #5 above) and both suggest that single doses of PGBx have no detectable effect on glucose metabolism on either control or "diabetic" animals. If so, the effect of PGBx observed by Dr. Polis in diabetic mice appears to be dependent upon the repeated treatments with PGBx over several weeks.

9. Brain Ischemia in Gerbils.

An attempt was made to develop an animal model for the effects of PGBx on brain ischemia using unilateral carotid artery occlusion in desert rats (Gerbils). This species is known to have a poorly developed circle of Willis and hence unilateral carotid artery ligation may be expected to result in significant but non-lethal brain ischemia. However pilot studies in a large number of Gerbils indicated that the results in control untreated animals were too variable and largely unpredictable to be used in test studies. This approach was therefore abandoned.

III. CONCLUSIONS:

The studies conducted under this contract provided the first demonstration that PGBx has a definite beneficial effect in vivo in promoting survival after a period of circulatory arrest. This was demonstrated in studies made in monkeys and dogs. Unfortunately attempts to demonstrate an effect of PGBx in more specific and/or a simple biological test system either in vivo or in vitro, were not successful. It is possible that the necessary conditions for demonstrating an effect of PGBx in the preparations tested were not achieved. Alternatively, it is also possible that the effects of PGBx in vivo depend on multiple mode action and therefore could not be demonstrated in more specific and/or simpler test systems.

In any event it should be emphasized that PGBx has an entirely novel biological action for which there is no existing biological test model beyond the original demonstration by Polis on isolated mitochrondria. Furthermore the condition for the demonstration of PGBx activity are rather unusual (e.g. it has no effect on normal tissues) and to a large extent critical, since it depends on a partially decompensated biological system which, however, still maintains the potential for recovery (i.e. availability of substrate as energy sources). In short PGBx has to be looked upon as a compound which facilitates and possibly re-couples energy utilization in a biological system but demonstration of its action depends on the availability of energy sources and carriers to effect recovery.

In view of this it is not surprising that the experimental conditions for a consistent demonstration of PGBx activity are very critical.

The availability of a high purity and uniform preparation of PGBx will make possible more extensive studies once the ambiguities of the possible effects of potential impurities, different polymer chains, etc. are eliminated or more fully controlled in the chemical preparation of this compound.

IV. PUBLICATIONS & REPORTS

Angelakos, E. T., R. West and E. Wudarski: Myocardial recovery after ischemia: In vivo effects of PGBx, a polymeric derivative of PGB_1. In: Advances in Prostaglandin and Thromboxane Research, Vol. 7, Ed: B. Samuelsson, P.W. Ramwell, and R. Paoletti. Raven Press, N.Y., 1980.

Angelakos, E. T., R. L. Riley and B. David Polis: Recovery of monkeys after myocardial infarction with ventricular fibrillation. Effects of PGBx. Physiol. Chem. & Physics 12:81-96, 1980.

V. ADDENDUM

Advances in Prostaglandin and Thromboxane Research, Vol. 7, edited by B. Samuelsson, P. W. Ramwell, and R. Paoletti. Raven Press, New York © 1980.

Myocardial Recovery After Ischemia: *In Vivo* Effects of PGB_x, A Polymeric Derivative of PGB_1

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The designation of prostaglandin B_x (PGB_x) was given by Polis to a compound he prepared from PGB_1 by a condensation reaction (5). On the basis of molecular weight and other studies, Polis suggested that PGB_x is a polymeric derivative of diketo-PGB_1 with a mean molecular weight of 2,200 (7). To avoid confusion it should be indicated that PGB_x is *not* the same compound originally designated as PG_x and subsequently identified as prostacyclin. Polis and associates found that when PGB_x was added in preparations of aged liver mitochondria *in vitro*, it restored oxidative phosphorylation and formation of adenosine triphosphate (5,7).

In previous studies made in our laboratory, we found that administration of PGB_x *in vivo* to monkeys subjected to periods of ventricular fibrillation (VF), in the presence of coronary ligation, had a significant effect in improving myocardial and general circulatory recovery. (1–3).

The studies to be reported here were conducted in dogs in an attempt to confirm and extend the previous findings in monkeys.

In the first series of experiments, the effect of PGB_x in promoting recovery after VF was tested. The protocol was similar to that used for the tests in monkeys. VF was induced electrically, and the animals were allowed to remain in this state of complete circulatory arrest for a period of 8 min. Resuscitation was then instituted by electrical defibrillation and cardiac massage. Just prior to resuscitation all animals received norepinephrine (500 μg) administered directly into the left ventricular cavity. Treated animals received in addition 10 mg of PGB_x in the same manner. Once circulation was established, the treated group received additional amounts of PGB_x (0.5–1 mg/kg, i.v.).

Of a total of 17 control animals tested, only 6 (35%) were successfully resuscitated after 8 min of VF. This is in general agreement with previous observations made in this and other laboratories. By contrast 6 of 7 (86%) of the PGB_x-treated animals recovered following defibrillation after the 8-min period of VF. The difference in survival rates is statistically significant ($p < 0.05$). These results of the effect of PGB_x in dogs are in general agreement with those previously obtained in this laboratory in monkeys.

Since the effect of PGB_x in favoring recovery after complete circulatory arrest with VF appeared to be primarily cardiac, additional studies were made to determine the effect of this compound on regional cardiac ischemia. In these studies, the descending branch of the anterior coronary was ligated near its origin and measurements of left ventricular pressure (LVP), rate of rise of LVP *(dP/dt)*, blood pressure, and cardiac output (CO) (by thermodilution technique) were made. This permitted assessment of overall left ventricular contractility (max *dP/dt*) and minute cardiac work (from CO and LVP). After 30 min, the ligation was released, and the same parameters were monitored for an additional period of 60 min. In these studies, treated animals received PGB_x (1 mg/kg, i.v.) just after the release.

Following ligation there was a 25–30% drop in CO. Release in both control and treated animals was generally followed by a further drop in CO within 10 min with partial recovery in the following 10 min. Subsequently, control animals showed a progressive deterioration, while the CO of PGB_x-treated animals showed no further decrease. Changes in calculated cardiac minute work were essentially parallel to those observed for CO.

The most striking differences between control and PGB_x-treated animals were observed in max *dP/dt*, reflecting overall left ventricular contractility. In this measurement also, the controls showed a further decrease following release. By contrast, the PGB_x-treated animals maintained the same level of *dP/dt* after release and for a period of 60 min thereafter. The difference between control and PGB_x-treated animals was statistically significant.

These and previous findings obtained by this laboratory from *in vivo* studies suggest that following a period of myocardial ischemia, administration of PGB_x preparations has a significant effect in restoring function and/or in preventing further functional damage of the myocardium. This effect could be related to the *in vitro* action of PGB_x in restoring oxidative phosphorylation in damaged mitochondria. Initial observations made in other laboratories suggest that PGB_x has a beneficial effect in certain other conditions involving ischemic injury (4,6).

It is noteworthy that no effect of PGB_x has been identified *in vivo* in the absence of a functional disarrangement, which is again consistent with the reported absence of any effect of PGB_x in normal undamaged mitochondria *in vitro*.

If all these *in vitro* and *in vivo* observations are related, PGB_x may represent a new class of pharmacologic agents which specifically promote functional recovery after an ischemic insult by acting at the mitochondrial level to restore oxidative phosphorylation. Such an action would represent a unique pharmacologic activity with extensive applications in a variety of pathologic conditions.

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RECOVERY OF MONKEYS AFTER MYOCARDIAL INFARCTION WITH VENTRICULAR FIBRILLATION. EFFECTS OF PGB_x

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• *PGB_x, a polymeric, stable, free radical derivative of 15-keto-prostaglandin B_1, that conserves oxidative phosphorylation in mitochondria under degenerative conditions* in vitro, *affected survival of male Rhesus monkeys (5-9 kg) anesthetized with pentobarbital and subjected to coronary ligation and induced ventricular fibrillation (VF). In tests performed in sequence with intervening periods for recovery, intracardiac injections of norepinephrine (NE), cardiac massage (CM), and electrical defibrillation (EDF) were used to restore cardiac function both in controls and experimental animals, but the latter were injected also with 1 mg/kg PGB_x. Recovery was established by maintenance of effective blood pressure without exogenous support. In the control group the cumulative survival for fibrillation episodes of 4, 6, 8, and 12 min was 60, 40, 31, and 25% respectively. In the PGB_x-treated group survival for equivalent periods was 100, 93, 93, and 88% respectively. In separate studies, African Green monkeys were subjected to single episodes of VF of either 8 or 12 min. Combined survival was 36% for the controls, 93% for the PGB_x-treated animals. Clearly PGB_x radically improved cardiac recovery after circulatory arrest due to VF in the presence of acute myocardial infarction. The results also suggest a synergistic action between norepinephrine and PGB_x in achieving such recovery.*

INTRODUCTION

Recovery following ventricular fibrillation (VF) in the presence of acute myocardial infarction is a well-recognized clinical problem, currently with no adequate effective therapy and a poor prognosis. Available evidence indicates that tissue ischemia leading to hypoxia and acidosis is associated with biochemical abnormalities involving insufficient energy for cellular survival because of decreased ATP production, mitochondrial damage, changes in membrane permeability, and lysosomal membrane rupture leading to irreversible cell damage and finally death.[1] Myocardial cell death is believed to occur when intracellular ATP drops below 2.0 μ moles/g in the perfused heart and the anaerobic metabolism of the ischemic heart cell stops.[2] In studies on ischemic anoxia produced by acceleration of rats at 20*g*, survival was markedly enhanced by drastic changes in pituitary-adrenal hormones which correlated with the maintenance of high levels of ATP in the brain.[3,4] In subsequent studies on degenerative biochemical changes in anoxic stress[5,6] a new bioregulatory factor was discovered with the unique property of conserving the mechanisms of oxidative phosphorylation of isolated mitochondria *in vitro* under degenerative conditions leading to a complete loss of the oxidative energy transformation process.[7,8] This factor, currently termed PGB_x, is a polymeric condensation of prostaglandin B_1 to form a new compound lacking the described properties of the parent prostaglandin.

When rat liver mitochondria are slowly

degenerated by aging at 0°C, followed by a brief Mg^{2+} catalyzed degeneration at 27°, complete loss of oxidative phosphorylation activity occurs. Addition of PGB_x to the reaction mixture preserves and restores oxidative phosphorylation to normal levels.[7,8]

In studies with inhibitors or Ca^{2+} competing for phosphorylation sites on the mitochondria, PGB_x acted to sustain oxidative phosphorylation. In the interplay between Ca^{2+} and PGB_x an *in vitro* control of the phosphorylation level could be achieved. All the effects of PGB_x were observed with so-called damaged mitochondria. No effect of PGB_x was observed with normal intact mitochondria. These findings suggested the use of PGB_x *in vivo* for the amelioration and survival of cellular catastrophes involving mitochondrial damage resulting in shock and death as seen in ischemic anoxia pathology.

The intent of the experimental design to be described was to investigate possible effect of PGB_x in the restoration of tissue and organ function after lethal periods of ischemia and hypoxia had rendered the organ intractable to the most effective therapeutic procedures known. Based on preliminary studies, the experimental procedure involved evaluation of overall cardiovascular recovery and survival of monkeys after a period of ventricular fibrillation in a heart with a left ventricular infarction from a coronary ligation. This provided an insult associated with a high incidence of mortality[9,10] and of such magnitude that recovery in untreated animals is at best difficult.

METHODS

Two species of monkeys, Rhesus (*Macaca mulata*) and African Green (*Cercopithecus aethiops*), were used. The monkeys were anesthetized with pentobarbital (30 mg/kg), then a thorocotomy on the left side between the 4th and 5th intercostal space was performed under positive pressure artificial respiration. Catheters were placed in the thoracic or abdominal aorta for direct recording of blood pressure and heart rate, and in the vena cava for venocylsis with Normasol, pH 7.4.* Lead I ECG was obtained with intradermal electrodes. Two stainless steel EEG electrodes were anchored 3 cm apart into the skull along the temporal ridge, positioned not to penetrate the dura. After stabilization, so that blood pressure was constant and the animal was able to maintain itself without assistance, the left anterior interventricular coronary artery was ligated just past the major branch approximately 1 cm from origin. In separate studies on Rhesus monkeys this ligation procedure caused an ischemic region involving an average of 27% of the left ventricular mass as measured by the radioactive microsphere technique.[11]

Coronary occlusion was utilized as part of the experimental design to assure that fibrillation once induced would be maintained. In initial studies in which VF was induced in animals without coronary occlusion there had been a high incidence of spontaneous defibrillation. Furthermore, the overall recovery after short periods of VF (4 or 8 min) was too high to permit an efficient assessment of any protective effect.

Arterial blood pressure, EEG, ECG, and heart rate were recorded for all animals. Myocardial segment tension, intraventricular pressure, dp/dt, and end-diastolic pressure as well as arterial blood gases, pH, and blood glucose levels were measured in selected animals. Since this latter information afforded little data correlative with recovery or death of the animal, it will not be referred to further in this paper.

Following coronary ligation, VF occurred

* Normasol R, pH 7.4, a preparation marketed by Abbott Laboratories, contains sodium chloride, 0.526%; sodium acetate, 0.222%; sodium gluconate, 0.52%; potassium chloride, 0.037%; and magnesium chloride, 0.014%.

spontaneously in approximately half the animals in the first 10 to 20 min. Incidence of spontaneous VF prior to 20 min was 56% in the controls and 44% in the PGB_x-treated group. The difference is not statistically significant. In those animals that did not fibrillate within the 20-min period after ligation, VF was induced electrically. VF was permitted to continue for specified time periods ranging from 4 to 24 min.

At the end of the prescribed period, resuscitation procedures were started consisting of (a) intracardiac injection of 500 μg norepinephrine (NE), (b) cardiac massage, and (c) electrical defibrillation. The latter was achieved with a DC defibrillator set to deliver 50-W pulses for 0.15 sec. The PGB_x-treated monkeys received the same resuscitation regime as the controls but with the additional intracardiac injection of 1 mg/kg PGB_x followed by cardiac massage and electrical defibrillation. All intracardiac injections were made into the left ventricular cavity. In addition, the treated animals received PGB_x (1 mg/kg) intramuscularly just prior to coronary ligation as well as additional doses of PGB_x (1 mg/kg) intravenously every 30 min throughout the experimental periods. PGB_x, prepared from 15-diketo PGB_1 methyl ester according to the method of Polis et al.[12] was administered as the sodium salt dissolved in Normasol R, pH 7.4, to a concentration of 10 mg/ml just before use.

Once the electrical and contractile activities of the heart were reestablished, the animal was allowed to recover spontaneously. If the animal remained in shock, NE (1-10 μg) was infused intravenously until the animal attained a blood pressure level over 40/20 mm Hg or became refractory to NE and died. If the monkey recovered from the first 4 min of fibrillation and became stable for a period of 20 to 30 min, it was subjected to the next longer fibrillation period of 6 min. In this manner animals were subjected sequentially to episodes of fibrillation of 4, 6, 8, and 12 min duration separated by 20-30 min recovery periods until the animal died in shock or successfully survived the course. The last group of animals, after recovering from 12 min of VF, was subjected to 24 min of VF.

Paired control and treated monkeys were run on the same day. When a control was run in the morning of one day and a treated animal in the afternoon, the order was reversed with the next pair. As far as possible, selection of the animals was random. In six instances, control monkeys that could not be brought out of shock with NE were then given PGB_x intravenously. Four out of six animals so treated revived to survive the sequential fibrillation series to 24 min.

To permit evaluation of the cardiovascular shock or recovery with PGB_x over a period of time after one ischemic event, another series of studies was made with African Green monkeys subjected to a single fibrillation episode of either 8 or 12 min. Recovery procedures were the same as with the sequential fibrillation studies except that the blood pressure levels in those animals that survived the initial fibrillation period were monitored for at least two hours. In general those animals that failed in shock did so within the first hour after defibrillation. This also permitted the determination of the ability of NE to maintain blood pressure levels in the presence or absence of PGB_x and thus evaluate the synergistic effects of PGB_x and NE.

For the *in vitro* studies, rat liver mitochondria were isolated by differential centrifugation in 0.3 M sucrose containing 5×10^{-4} M EDTA pH 7.4. The isolation and assay methods employed standard techniques.[13] Mitochondrial preparations used contained little lysosomal activity as evidenced by measurements of acid phosphatase activity, which was less than 2% of that found in whole liver.[14] Addition of PGB_x

did not alter the acid phosphatase activity in these preparations. Mitochondria were stored in 0.3 M sucrose containing 5×10^{-4} M EDTA at a concentration of 100 mg protein/ml at 0°C until used. Although the PGB_x effect on damaged mitochondria could be shown with first day preparations, 3-5 days of aging at 0°C normally was required to demonstrate the maximum PGB_x effect. On the other hand, some rat liver mitochondria preparations were found to be active even after 10 days at 0°C. For the electron microscopy studies of freshly isolated rat liver mitochondria, samples were centrifuged at 6000*g*, the sucrose removed and the pellet layered with 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. At 30-min intervals the mixing reagent was changed 3 times. For the control and PGB_x experiments, 4 mg of 5-day-old mitochondria were incubated in a mixture containing 0.1 ml of 0.1 M potassium phosphate buffer, pH 7.4; 0.16 ml of 0.20 M sodium α-ketoglutarate, pH 7.4; and 0.1 ml of 0.1 M $MgSO_4$, in a total vol of 2.01 ml for 15 min in a shaker bath at 27°C. In the PGB_x experiments 10 μg of PGB_x was added to the above mixture. At the end of the preincubation period, the assay for phosphorylation was begun by the addition of 0.15 ml of a mixture of 0.05 ml of 0.1 M ADP, 0.05 ml of 0.1 M AMP, and 0.05 ml of 2 M KCl, followed immediately by the addition of 0.04 ml of 3.75% solution of crystalline bovine serum albumin to give a final mixture of 2.2 ml. AMP and ADP were added as phosphate acceptors, KCl was added to maintain tonicity, and serum albumin for

TABLE I. Survival of Control and PGB_x-Treated Monkeys Subjected to Incremental Periods of Fibrillation after Left Anterior Coronary Artery Ligation

Control							PGB_x-treated						
No.[a]	Fibrillation (min)						No.	Fibrillation (min)					
	4	6	8	12	24			4	6	8	12	24	
18 C	F[b]						14 E		F				
31 C	F						15 E		S	S	S		
35 C	F						19 E	S	S		S		
37 C	F						20 E	S	S	S	S		
32 C	S[c]	F					21 E	S	S	S	S		
33 C	S	S	F				22 E	S	S	S	S		
24 C	S	S	S	S			23 E	S	S	S	S		
45 C	S		F				36 E	S	S	S	F		
11 C		F					38 E	S	S	S	S	S	
12 C		F					39 E	S	S	S	S	F	
13 C		S	S	S			40 E	S	S	S	S	S	
16 C		S	S	F			41 E	S	S	S	S	S	
17 C		F					42 E	S		S	S	S	
26 C		F					43 E	S		S	S	S	
25 C	S	S	S	S	F		27 E	S	S	S	S		
44 C	S		S	S	S		29 E	S	S	S	S		
	Cumulative % survival												
	60	40	31	25				100	93	93	88		

[a] Refers to identification number of individual monkey. [b] (F) indicates monkey failed to recover from the fibrillation and sustain a blood pressure above shock. [c] (S) indicates successful recovery out of shock for the trial period.

binding fatty acids released during the degeneration of the mitochondria. This sequence for the addition of reactants was found to be optimal for demonstrating the PGB_x effect.

In order to obtain enough material for the EM experiment, five control vessels and 5 vessels containing PGB_x were reacted with constant shaking for 20 min. The reaction vessels were pooled in ice-cold tubes and centrifuged at 10,000*g* for 10 min. The supernatants were removed and analyzed for inorganic phosphate remaining[15] in order to check the effectiveness of the PGB_x preparation. The pellets were then fixed with cold buffered glutaraldehyde as described before.

The fixed pellets from the freshly isolated mitochondria from control and PGB_x reacted mitochondria were minced with a freshly degreased razor blade, and the resulting segments were immersed in Millonigs-phosphate buffer (MPB) and fixed further with 1.0% OsO_4 in the MPB for one hour. Fixation was followed by rinsing in several changes of MPB, and dehydration was accomplished in a graded series of alcohol solutions (30, 60, 90, 100, and again 100%) followed by 2 changes in propylene oxide, impregnated and embedded in epoxy. Thin sections of the epoxy-embedded mitochondria, cut with a Sorval MT2B ultramicrotome fitted with a diamond knife, were post-stained with uranyl acetate and lead citrate.

For the electron microscopic studies of monkey heart tissue, the aorta was cannulated and the beating heart was subjected to retrograde perfusion at a pressure of 110 cm H_2O initially with buffered saline and subsequently to 1.25% glutaraldehyde buffered with 0.08 M sodium cacodylate and 0.03 M $CaCl_2$ (pH 7.4).[16] Small tissue samples were obtained from normal and ischemic regions; they were immersed in the same fixative for one-half hour. Tissues were further trimmed to segments measuring about 0.5 mm^3 and post-fixed in 1.0% OsO_4 (0.1 M cacodylate with 3.0% sucrose) at 4°C and brought to room temperature for a total post-fixation period of one hour. The tissue, after being rinsed twice in 0.2 M cacodylate buffer dehydrated in a graded series of alcohol solutions, was treated with propylene glycol prior to embedding in epoxy as described above. For each study, 9 electron images of each of 6 examples were recorded to electron optical magnifications of 3400× and 8200× employing an RCA EMU-4 electron microscope. All the EM observations and interpretations were made by Dr. John T. Stasny.

RESULTS

Studies in Rhesus Monkeys

Initial fibrillation. Table I summarizes the results of the survival of Rhesus monkeys subjected to periods of fibrillation. A total of 10 control and 14 PGB_x-treated animals were subjected to an initial episode of VF of 4-min duration. At the end of this period, 6 of the controls and all 14 of the PGB_x-treated animals recovered. This difference is statistically significant at $p < 0.02$ (Fisher's exact test).

Sequential fibrillation. Since all of the PGB_x-treated animals survived the initial tests of 4 min, the studies were extended by subjecting all survivors (control and treated) to additional periods of VF. After the initial episode, animals were allowed to recover and were subjected to progressively longer periods of VF of 6, 8, 12, and 24 min. As shown in Table I, the cumulative survival in the controls decreased from 60% at 4 min to 25% after the 12-min episode. In the PGB_x-treated group, the 100% survival rate after 4 min of VF was maintained at 88% after the sequential exposure to 6, 8, and 12 min of VF. This difference is sta-

tistically significant at $p < 0.01$ (chi-square test). Furthermore, these results indicate that while survival in the controls was decreasing progressively with the longer periods of VF as may be expected, the PGB_x-treated group maintained a high survival rate in the successive tests. This provides additional evidence that the differences obtained in the initial tests were not due to chance.

Six of the PGB_x-treated animals that survived the 12-min period were exposed to 24 min of VF. Of these, 5 survived. Of the 4 controls that survived 12 min of VF, 2 were tested at 24 min. One survived. Specific statistical comparisons of the survivors after 24 min of VF is not possible since not all the animals surviving the 12 min of VF were tested at 24 min (Table I).

Defibrillation was achieved with a single shock in most of the PGB_x-treated monkeys (12 of 14) and about half the control monkeys (6 of 10). A few controls required 2 to 3 shocks. In general, electrical defibrillation was uniformly successful and was not a significant factor in survival. The major difference between the PGB_x-treated and control animals was, that in the former the recovery of cardiac contractile force following electrical defibrillation was more rapid and more complete. Control animals in which cardiac contractile activity did not recover sufficiently to maintain an adequate level of blood pressure often developed recurrent episodes of fibrillation and/or cardiac arrest during the resuscitation phase. In general, such animals did not recover. These observations on untreated animals are similar to those observed in other species (dogs, pigs) in this and other laboratories. There was, however, a difference in the incidence of spontaneous defibrillation. Many of the PGB_x-treated animals defibrillated spontaneously and required repeated electrical interventions to maintain the fibrillation for the experimental period. This was rarely observed in the controls. Table II summarizes the frequency of spontaneous defibrillation in control and PGB_x-treated monkeys.

TABLE II. Spontaneous Recovery from Ventricular Fibrillation After Induced Myocardial Infarction in Rhesus Monkeys

Fibrillation time (min)	Incidence of spontaneous defibrillation (%)	
	Controls	PGB_x-treated
4	0	8
6	7	34
8	7	39
12	0	29
Mean:	3.7	28

Studies in African Green Monkeys

Initial VF. Studies involving a single prolonged episode of VF were made on 14 control and 14 PGB_x-treated African Green monkeys. Two different groups of animals were exposed to a single episode of either 8 or 12 min of VF. The results, summarized in Table III, were simliar to those obtained in the sequential VF studies with Rhesus.

Of those Greens exposed for 8 min, 89% of the PGB_x-treated group recovered and 33% of the controls recovered. Of those exposed for 12 min, 100% of the PGB_x-treated group recovered and 40% of the control group recovered. The differences are statistically significant ($p < 0.05$). The combined survival (8 and 12 min) was 93% for the treated as compared to 36% for the controls ($p < 0.01$).

This experimental design, involving a single episode of VF, made possible extended observation on the cardiovascular status of the animal for a period of 2-3 hours after VF. It was noted that a significant number of control animals, that had recovered cardiac activity after initial resuscitation rapidly deteriorated into a state of circulatory shock.

TABLE III. Survival of Control and PGB_x-Treated African Green Monkeys after a Single Fibrillation Episode

VF period (min)	Control		PGB_x-treated	
	No. surviving / Total tested	% Survival	No. surviving / Total tested	% Survival
8	3/9	33	8/9	89*
12	2/5	40	5/5	100*
Total	5/14	36	13/14	93†

* $p < 0.05$. † $p < 0.01$.

During this period, repeated administrations of NE to these controls resulted in short-lived pressor effects that became progressively less (and/or required higher doses of NE) until no effect could be obtained with even very large doses of NE. In contrast, many PGB_x-treated animals responded to NE with a pressor effect that did not return to the previous baseline. Thus in the PGB_x-treated group, progressively smaller doses of NE were needed to maintain adequate blood pressure, and eventually the pressure was maintained without any exogenous NE.

Infusions of catecholamines after resuscitation. To evaluate this phenomenon more directly, experiments were carried out in which animals were infused continuously with NE following a period of VF, and the infusion rate was adjusted to maintain a diastolic pressure of 60 mm Hg during the post-fibrillation period. The doses of NE required to achieve this ranged from 0.3 to 300 μg/min. Six control and 12 PGB_x-treated African Green monkeys were studied after the initial resuscitation. In 5 out of 6 controls (83%) the amount of NE infused had to be progressively increased to maintain the desired pressure. In 4 of these animals the pressure could not be maintained and the animals died. By contrast, in 7 out of 12 PGB_x-treated animals the amount of NE infused necessary to maintain 60 mm Hg was progressively decreased, and all 12 animals survived.

In other experiments with control animals whose blood pressure was at shock levels, the administration of repeated doses of NE did not produce a pressor effect. When these animals were treated with PGB_x, subsequent administration of NE at the previously ineffective dose levels usually produced distinct pressor responses.

Catecholamines and PGB_x. In initial studies it was found that intracardiac administration of NE during the resuscitation period improved greatly the incidence of recovery and the subsequent status of the animal in both control and PGB_x-treated preparations. Therefore intracardiac NE was included as a standard measure in the studies reported here. Observations made during these initial and subsequent studies suggested a potentiating effect between PGB_x and catecholamines. To determine whether PGB_x potentiated any of the cardiovascular effects of NE in normal animals, 3 normal anesthetized monkeys (Green) were investigated for dose-response pressor and cardio-accelerator effects of NE (2-5 μg/kg) before and after PGB_x. These did not demonstrate any potentiating effect. However, in the animals with blood pressure at shock levels, pretreatment with PGB_x produced a distinct potentiation of the pressor actions of NE. An example is shown in Fig.

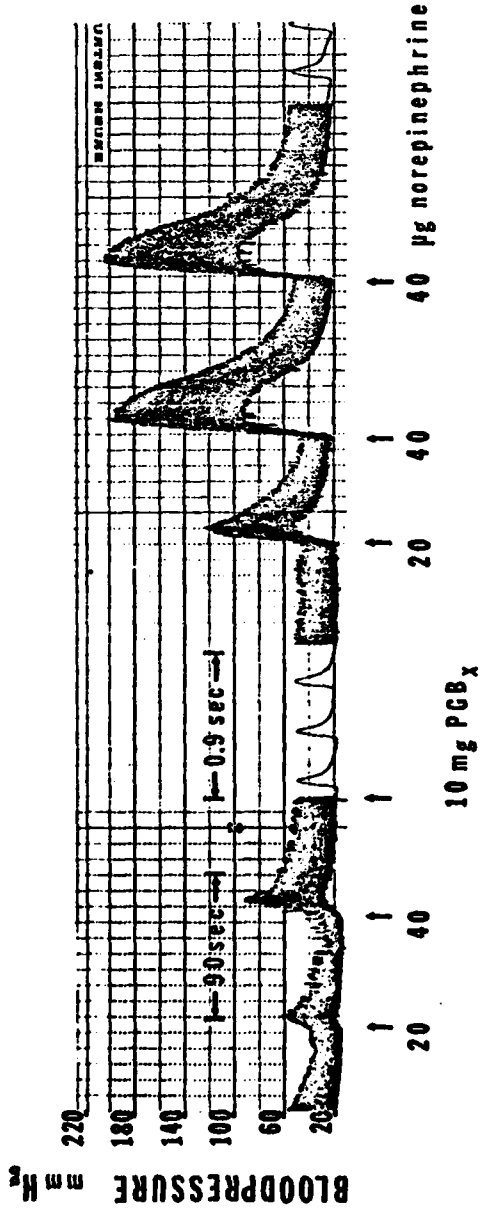


FIGURE 1. Potentiation of the pressor effect of norepinephrine (NE) following treatment with PGB_x. This animal had shock levels of pressure and negligible responses to 20 and 40 µg doses of NE. The same doses of NE produced marked pressor responses when tested within 3 to 5 min after administration of 10 mg of PGB_x. Blood pressure scale: 20 mm Hg/div. Time scale: 10 sec/div (slow record) and 0.1 sec/div (fast record).

1. Unfortunately this effect was evident only in the presence of severe hypotension (below 40 mm Hg), was not consistent, and/or the conditions necessary to reproduce it could not be fully identified. Hence it was not possible to obtain quantitative information on the potentiation, nor was it feasible to study the effect of adrenergic blocking agents.

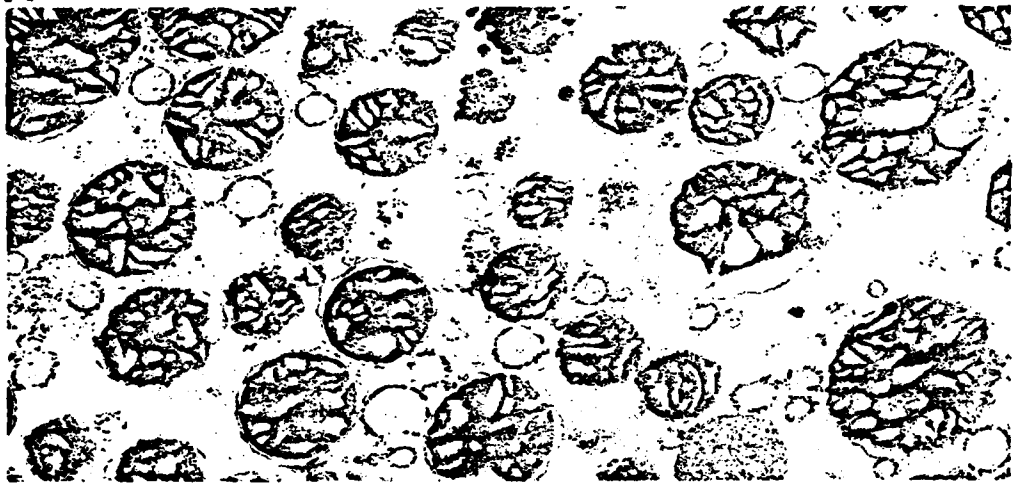
Electron Microscopic Studies

In vitro studies. In an effort to complement the kinetic studies *in vitro,* and the recovery studies *in vivo* with correlative changes in mitochondrial structure, electron microscopy was carried out on isolated mitochondria subjected to degenerative conditions in the absence and presence of PGB_x. These are compared with the sections of heart from areas considered normal and from the infarct area of control and PGB_x-treated monkeys after fibrillation for 12 min followed by the resuscitation procedures.

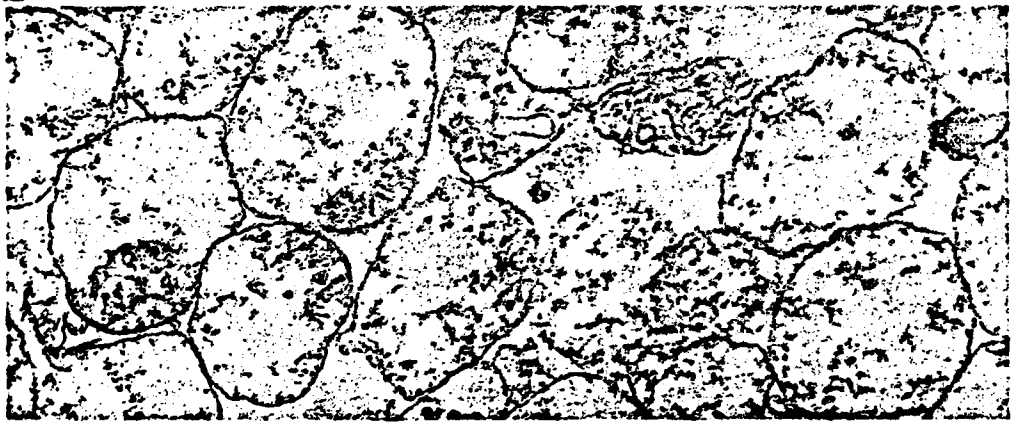
The EM observations made in the 3 mitochondrial preparations are illustrated in Fig. 2. Figure 2A represents normal 6-hour-old

FIGURE 2. Transmission electron microscopy observations on isolated rat liver mitochondria (20,000 ×). (A) These are 6-hour-old mitochondria of excellent homogeneity. Almost all are in state III or the condensed configuration that represents the low energy state of isolated rat liver mitochondria. The matrix material is very dense. Only a few mitochondria are not in the condensed state. Some microsomes also can be seen. (B) These are 5-day-old mitochondria isolated after preliminary degeneration and 20-min reaction as described under METHODS. All are swollen 2 to 3 times the size of the 6-hour-old mitochondria but do not appear lysed and show only a very small amount of granular intramitochondrial content and almost no remnants of cristae. (C) These are 5-day-old mitochondria treated with PGB_x and isolated after preliminary degeneration and 20-min reaction as described under METHODS. The PGB_x-treated mitochondria are less swollen than the untreated mitochondria (B) and contain more matrix material and membranous derivatives of cristae.

A



B



C

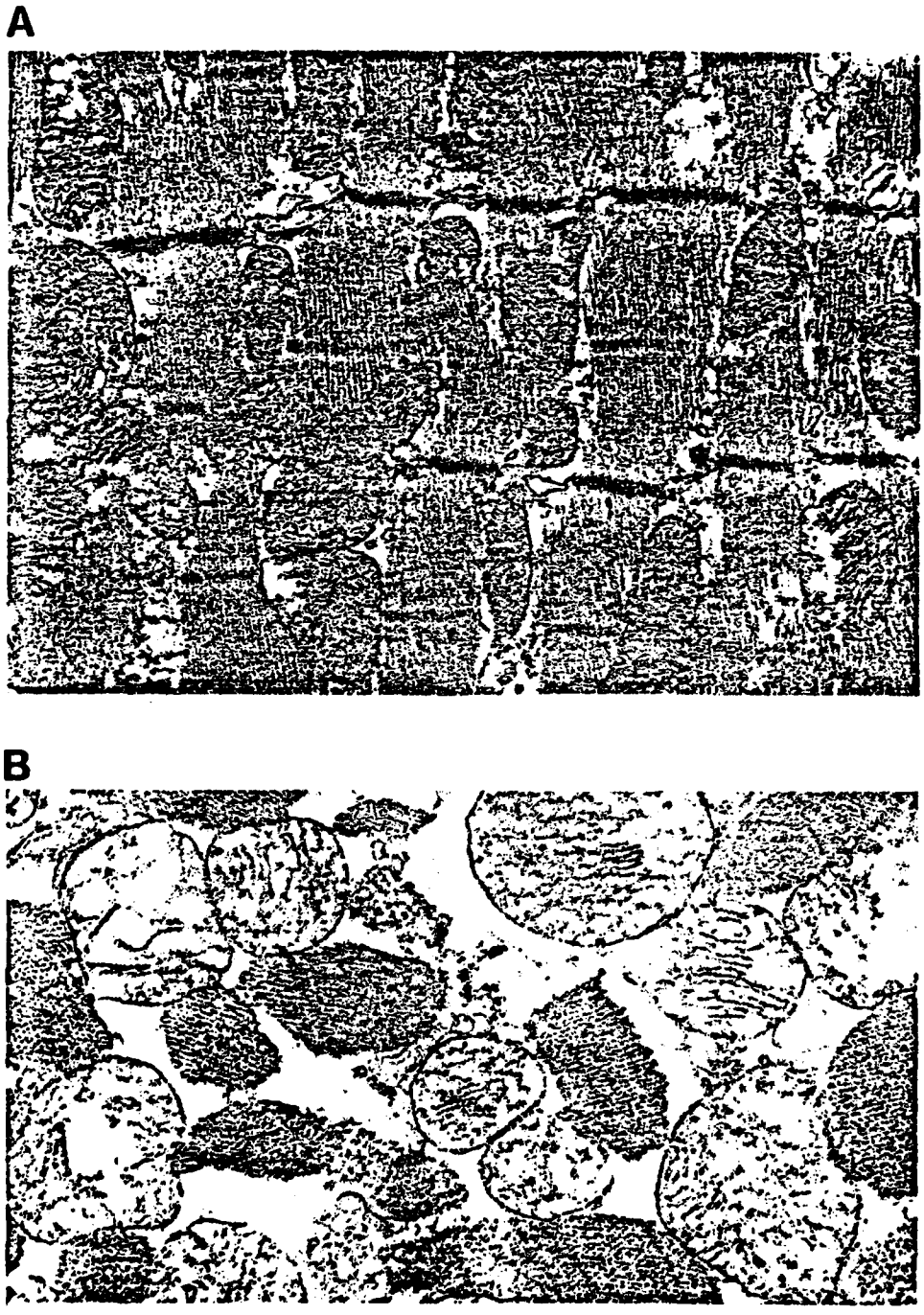


FIGURE 3. Transmission electron microscopy observations on tissue sections from an untreated monkey with myocardial infarction after 12 min of VF (24,600 ×). (A) Noninfarcted tissue from base of left ventricle. In noninfarcted region, mitochondria in some areas are well preserved while in other areas they are lacking in matrix density but show numerous intact and prominant cristae. (B) Center of infarcted area. Mitochondria are swollen, disorganized, and deteriorated. Cristae are in short segments within a leached matrix.

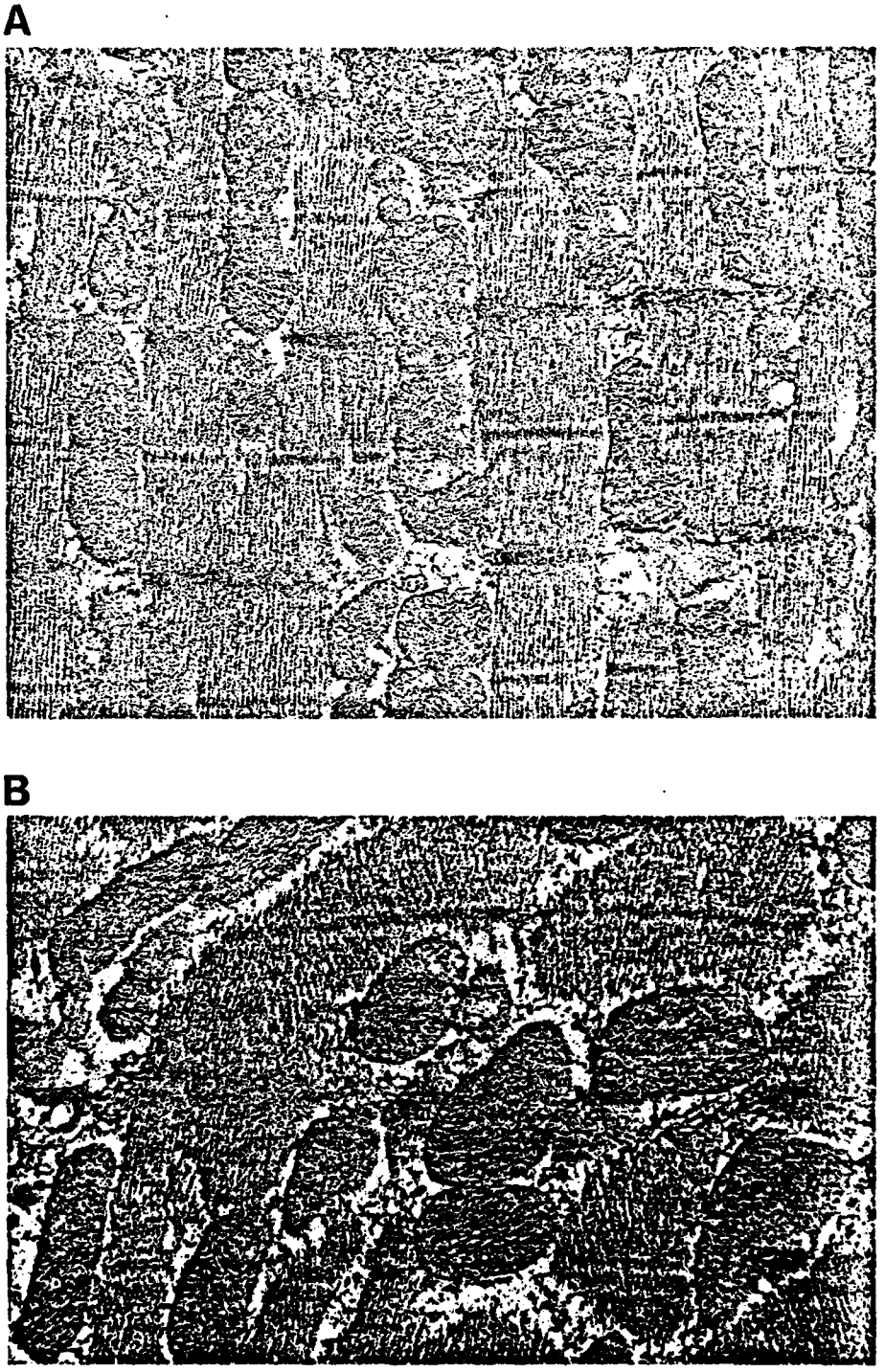


FIGURE 4. Transmission electron microscopy observations on tissue sections from a monkey treated with PGB_2 after 12 min of VF (24,600 ×). (A) Noninfarcted tissue from base of left ventricle. Mitochondria matrix in general is dense and in good condition. (B) Center of infarcted area. Mitochondria are numerous and small; some show unusual shapes but are nevertheless intact and dense.

mitochondria showing the intact and homogenous condition of the original isolated preparation. This is consistent with the high phosphorylation ability of this preparation (6 μmoles of inorganic phosphate esterified per 4 mg mitochondria for the 20-min reaction period). Figure 2B shows mitochondria degenerated for 15 min and reacted under conditions of oxidative phosphorylation. This preparation, which esterified only 0.32 μmoles of inorganic phosphate, served as the control. The mitochondria in the EM of Fig. 2C are similar to the control (2B) except that 10 μg of PGB_x were added to the reaction mix. In the presence of PGB_x, oxidative phosphorylation was conserved and the mitochondria esterified 4.36 μmoles of inorganic phosphate over the same time which is equivalent to 73% of the maximum phosphorylation obtained with freshly isolated mitochondria. Details of the EM differences are outlined in the legend of Fig. 2.

In vivo studies. Tissues for the electron microscopic studies were removed from 3 untreated and 3 PGB_x-treated monkeys. In these studies the animals were subjected to single periods of VF of 12 min and in all cases VF was induced 20 min after ligation.

Sections were taken from the left ventricle, thus: (a) from a distant, apparently undamaged area in the base of the left ventricle and (b) from the middle of the infarcted region. Representative sections are shown in Figs. 3 and 4. Tissues shown in Figs. 3A and 3B were taken from an untreated monkey after a 12-min period of VF once it was established that the animal had failed to recover. The PGB_x-treated animal (Figs. 4A and 4B) was also exposed to 12 min of VF, but it survived and was sacrificed after a 2-hour monitoring period. In general, the mitochondrial tissues from PGB_x-treated animals were in good condition. Although many mitochondria in the infarcted area show bizarre elongated shapes, their matrix density and the good condition of membranes suggest structural integrity. This differs considerably from the degenerated, vacuolated condition of the mitochondria from equivalent regions of the untreated animal hearts (Fig. 3B). Tissue samples from the "border" zone were also studied. These showed similar differences between control and treated animals. In view of this, and considering the ambiguities of sampling from the border zone, these studies did not provide any additional information and are not included in this report.

For our purposes morphometric considerations were of less concern than the condition of the mitochondria, the organelles targeted for demonstrating the response to treatment. Hence quantitative data were not recorded. Similarly, no attempt was made to preferentially orient the tissue fibers during preparation for EM to show sarcomeres in comparable displays. The fixation procedure used was that of Tomanek and Karlsson,[16] which is considered to be optimal. However, it should be emphasized that all the tissues studied, including those obtained from non-ischemic regions, were subjected to periods of ischemia and hypoxia during the periods of induced VF. Alterations in tissue structure would therefore be expected. In view of this, the preserved state of the mitochondria of the treated animals is to be considered remarkable.

DISCUSSION

The primary hypothesis underlying the described experiments is that since PGB_x has a unique *in vitro* action in the conservation of oxidative phosphorylation under conditions degenerative to mitochondria, a similar action *in vivo* should serve to enhance survival of an ischemic anoxic crisis incurring mitochondrial damage. This implies that car-

diac muscle contraction is limited by the rate at which chemical energy can be supplied by the metabolic process. Although there is ample evidence[1] for biochemical and morphological changes in the infarcted myocardium with considerable damage in the forms of vacuolation, swelling, and loss of structure seen in mitochondria, it is important to remember that a distinction exists between biochemical and structural damage. Mitochondria that may take a long time to recover morphologically seem to regain and maintain their functional integrity even though they appear ragged and disrupted.[17] Despite these elements of uncertainty for specific sites of dysfunction, there remains the overall failure in the mechanism coupling energy transformation with energy utilization. It is in this mechanism that we propose a role for PGB_x, which reacts synergistically with norepinephrine to reestablish the flow of energy to the contractile process in cardiogenic shock. PGB_x can be definitively associated with conservation and reactivation of mitochondrial synthesis of ATP from the *in vitro* findings.[7,8] According to Ellis,[18] the evidence for norepinephrine action on contraction seems to be that it takes place at some site coupling metabolic energy to the contractile process. Catecholamines simultaneously increase ATP breakdown and contractile force.[19] Adrenergic mediators also increase the maximum velocity of shortening.[20,21] The combined action of PGB_x and norepinephrine then could reestablish both sufficient energy and sufficient utilization to account for survival after the ischemic crisis.

The repeatedly confirmed findings that PGB_x has no effect or even a small inhibitory action in intact mitochondria *in vitro* and that it has no demonstrable effect on the normal animal cannot be overemphasized. The dramatic actions of PGB_x occur only with damaged mitochondria *in vitro* or after ischemic pathology *in vivo*. The effects observed suggest the PGB_x can replace or bridge an essential factor in energy transformation that is lost when mitochondria are swollen and damaged.

The results obtained in the studies of both Rhesus and African Green monkeys indicate that treatment with PGB_x greatly improves the incidence of survival after periods of complete circulatory arrest produced by VF. This conclusion can be based primarily on the results attained with the initial tests of VF used in Rhesus monkeys and with the single spisodes of VF used in African Green monkeys. Considering both species, the survival after single or initial episodes of VF of 4, 8, and 12 min was 60%, 32%, and 29% in the controls and 100%, 92%, and 90% in the PGB_x-treated. Studies with the repeated sequential VFs performed in the Rhesus monkeys provide additional confirmation that the differences in the initial episodes were not due to a chance occurrence, even at a low probability. If the higher survival to the first test were due to a chance occurrence, survivals to subsequent progressively longer results would be expected statistically to show an exponentially decreasing rate of cumulative survival, as was found in the case of the controls. By contrast, the PGB_x-treated group has a relatively unchanged rate of survival over the period tested, providing strong statistical evidence that the initial result obtained was not due to a chance occurrence. The cumulative test results also show that the median survival time (for 50% survival) after VF was increased from between 4 to 6 min in the controls to more than 12 min in the PGB_x-treated animals. Recovery after circulatory arrest depends initially on the restoration of electrical and mechanical activity of the heart and subsequently on the reestablishment of the cardiovascular control mechanisms responsible for the maintenance of effective blood pressure levels. Improved

recovery is therefore likely to involve primarily cardiac effects followed by reversal of the shock state.

The experimental methods used in the present studies included prior insult to the heart by coronary ligation involving a significant proportion of the left ventricle. Under these conditions improved cardiac resuscitation could be the result of improvements of (a) the general status of the entire heart, or (b) primarily the marginal ischemic regions.

It is well known that cardiac resuscitation is more difficult in the presence of coronary occlusion.[9,10] Therefore one possible interpretation of the results is that treatment with PGB_x in some way alters the degree of myocardial injury associated with coronary occlusion. This could be the case if PGB_x reduced the size of the metabolically injured myocardium following coronary occlusion. It is now generally believed that tissue injury produced by coronary occlusion includes a significant portion of marginal tissue with diminished blood flow, the ultimate fate of which depends upon the discrepancy between the tissue metabolic demands and the reduced circulation.[22-24] The effect of PGB_x in restoring phosphorylating activity of previously damaged mitochondria *in vitro* is consistent with the possibility that the results of the present study could be due to PGB_x actions on the marginal areas of coronary occlusion. However, considering that VF produces generalized hypoxia in the entire heart, an equally likely action of PGB_x is that it affects the ability of the entire heart to recover. Most likely, both factors play a significant role in determining the difference in the survival between control and PGB_x-treated animals in the present experiments.

Alternative interpretations of the results also should be considered. It is possible that the observed effect of PGB_x in the intact animal is unrelated to its actions on isolated mitochondria and that it represents unrecognized effects on the sarcoplasmic reticulum or other cellular membranes such as lysosomes. Moreover the *in vitro* effect of PGB_x in favoring phosphorylation to Ca^{2+} uptake may have its counterpart in regulating excess Ca^{2+} in the contractile process of the anoxic myocardium. It is also possible that the observed *in vivo* effect may depend on the interaction between PGB_x and other naturally occurring compounds. In this connection the potential interaction between PGB_x and circulating catecholamines is of particular interest in view of the observations made during the present studies on the biological interactions between PGB_x and norepinephrine. All these alternates represent plausible speculations subordinate to the firm experimental evidence of PGB_x action on mitochondria.

Although PGB_x is a polymeric derivative of prostaglandin B_1, it exhibits none of the reported activities of any of the known prostaglandins. Both its molecular size and structure favor a unique metabolic action not shown by the monomeric prostaglandins. It is also unrealistic to expect that PGB_x would be converted metabolically to a monomeric prostaglandin. Therefore it would be unlikely to have biological properties attributed to the known prostaglandins. PGB_x has no structural similarity to PGX (PGI_2 or prostacyclin) recently identified.[25,26] It should be noted that PGB_x has none of the cardiovascular actions reported for prostacyclin, such as systemic vasodilation and reduction of blood pressure, nor is there any evidence that PGB_x shares any of the cellular or antithrombotic effects of prostacyclin. Therefore the suggested actions of prostacyclin on myocardial infarction[27] may be basically quite distinct from those described here for PGB_x.

Similarly, recent reports on the effects of monomeric prostaglandins on the ischemic myocardium[28-30] appear to involve a different mechanism of action than that of

PGB_x. Furthermore, none of the single prostaglandins have an effect on mitochondrial phosphorylation *in vitro* comparable to that of PGB_x. On the other hand, it is possible that the PGB_x effects *in vivo* may be related to some as yet unidentified activity perhaps shared to a greater or lesser extent by other prostaglandins. In any event, more recent studies with PGB_x in other experimental preparations involving tissue ischemia *in viv* have confirmed a beneficial effect of this compound in promoting subsequent recovery.[31,32]

In general the present results favor the view that PGB_x has an activity *in vivo* similar to that previously demonstrated in isolated mitochondria. PGB_x then would constitute the prototype of an entirely new class of compounds whose biological activity would involve restoration of metabolic functions following hypoxic or ischemic injury. Pharmacological compounds possessing such an activity would have a broad application in a variety of diseases and traumatic states.

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